REGUIVED CENTRAL FAX CENTER

LISTING OF THE CLAIMS:

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The listing of the claims below replaces all prior versions of the claims.

- 1. (Currently Amended) A method for purifying a polyhistidine-tagged cytokine from a protein preparation derived from a mammalian cell culture, wherein the polyhistidine-tagged cytokine is present in the protein preparation at a concentration of no more than 2mg/L, said method comprising:
 - (a) concentrating the polyhistidine-tagged cytokine in the protein preparation with a negatively charged capture support, wherein the negatively charged capture support comprises heparin, comprising the steps of:
 - (i) contacting the protein preparation with the capture support;
 - (ii) washing the capture support with a capture support washing buffer of low ionic strength to remove interfering molecules but not the polyhistidine-tagged cytokine from the capture support; and
 - (iii) eluting the polyhistidine-tagged cytokine from the capture support with a capture support eluting buffer of high ionic strength;
 - (b) purifying the polyhistidine-tagged cytokine from the eluate of step (a) (iii) with a tag-specific affinity support, wherein the affinity support comprises nickel nitrilotriacetic acid immobilized on a solid support, comprising the steps of:
 - (i) contacting the eluate of step (a) (iii) with the tag-specific affinity support;
 - (ii) washing the affinity support with affinity support washing buffer of low ionic strength to remove some impurities but not the polyhistidine-tagged cytokine from the affinity support; and
 - (iii) eluting the polyhistidine-tagged cytokine from the affinity support with an affinity support eluting buffer.
- 2. (Currently Amended) The method of claim 1, wherein the capture support washing buffer and the affinity support washing buffer comprise an ionic strength equivalent to <u>from</u> about 50 mM to about 150 mM salt equivalent.
- 3. (Original) The method of claim 2, wherein the capture support eluting buffer comprises an ionic strength equivalent to at least about 500 mM salt equivalent.

- 4. (Original) The method of claim 3, wherein the capture support is applied to a column before or after contacting with the protein preparation.
- 5. (Original) The method of claim 3, wherein the affinity support is applied to a column before or after contacting with the eluate of the capture support.

6.-9. (Canceled)

- 10. (Previously Presented) The method of claim 3, wherein the affinity support eluting buffer comprises at least 50mM imidazole.
- 11. (Previously Presented) The method of claim 10, wherein the polyhistidine-tagged cytokine is a 6x histidine tagged cytokine with a four-helix bundle motif.

12.-16. (Canceled)

- 17. (Currently Amended) A method for purifying a polyhistidine-tagged cytokine with a four-helix bundle motif from a protein preparation derived from a mammalian cell culture, wherein the polyhistidine-tagged cytokine is present in the protein preparation at a concentration of no more than 2mg/L, said method comprising:
 - (a) concentrating the polyhistidine-tagged cytokine in the protein preparation with a negatively charged capture support, wherein the negatively charged capture support comprises heparin, comprising the steps of:
 - (i) contacting the protein preparation with the capture support;
 - (ii) washing the capture support with a capture support washing buffer of an ionic strength equivalent to a concentration of about 50 mM to about 1 M to remove interfering molecules but not the polyhistidine-tagged cytokine from the capture support; and
 - (iii) eluting the polyhistidine-tagged cytokine from the capture support with a capture support eluting buffer of an ionic strength equivalent to a concentration of about 50 mM to about 1 M;
 - (b) purifying the polyhistidine-tagged cytokine from the eluate of step (a) (iii) with a tag-specific affinity support, wherein the affinity support comprises nickel nitrilotriacetic acid immobilized on a solid support, comprising the steps of:

- (i) contacting the eluate of step (a) (iii) with the affinity support;
- (ii) washing the affinity support with affinity support washing buffer of an ionic strength equivalent to a concentration of about 50 mM to about 1 M to remove some impurities but not the polyhistidine-tagged cytokine from the affinity support; and
- (iii) eluting the polyhistidine-tagged cytokine from the affinity support with an affinity support eluting buffer comprising at least 50 mM imidazole.

18.- 24. (Canceled)

- 25. (Previously presented) The method according to claim 1, wherein the polyhistidine-tagged cytokine is a polyhistidine-tagged human cytokine.
- 26. (Previously presented) The method according to claim 25, wherein the human polyhistidine-tagged human cytokine is polyhistidine-tagged IL9ra.
- 27. (Previously presented) The method according to claim 17, wherein the polyhistidine-tagged cytokine is a polyhistidine-tagged human cytokine.
- 28. (Previously presented) The method according to claim 27, wherein the human polyhistidine-tagged human cytokine is polyhistidine-tagged IL9ra.
- 29. (New) The method of claim 1, wherein the protein preparation is derived from a mammalian cell culture supernatant or a mammalian cell culture cell lysate.
- 30. (New) The method of claim 29, wherein the protein preparation is derived from a mammalian cell culture supernatant.
- 31. (New) The method of claim 30, wherein the polyhistidine-tagged cytokine is transiently expressed in the mammalian cell culture.
- 32. (New) The method of claim 30, wherein the polyhistidine-tagged cytokine is nearly 100% captured from the protein preparation with greater than 99% purity.

- 33. (New) The method of claim 17, wherein the protein preparation is derived from a mammalian cell culture supernation or a mammalian cell culture cell lysate.
- 34. (New) The method of claim 33, wherein the protein preparation is derived from a mammalian cell culture supernatant.
- 35. (New) The method of claim 34, wherein the polyhistidine-tagged cytokine is transiently expressed in the mammalian cell culture.
- 36. (New) The method of claim 34, wherein the polyhistidine-tagged cytokine is nearly 100% captured from the protein preparation with greater than 99% purity.